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The effect of *Pistacia terebinthus* extract on lipid peroxidation, glutathione, protein, and some enzyme activities in tissues of rats undergoing oxidative stress

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Abstract: This study measures the effects of *Pistacia terebinthus* flower extract on the amounts of lipid peroxidation (LPO), total protein, glutathione, and enzyme activities in blood. It also measures the effects of the plant extract on glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) enzyme activities and it arrives at the conclusion that GSH-Px activity significantly increased in the *Pistacia terebinthus*-hydrogen peroxide group ($P < 0.0001$) and SOD activity meaningfully increased in the *Pistacia terebinthus* ($P < 0.05$) and *Pistacia terebinthus*-hydrogen peroxide ($P < 0.0001$) groups. It is also found that *P. terebinthus* extract has protective effects against LPO exerted in the kidney, brain, and lungs ($P < 0.0001$). The positive effects of *P. terebinthus* extract are also found on the levels of glutathione and total protein in all organs, except for the level of glutathione in the spleen and brain, and the increase of glutathione and total protein ($P < 0.0001$). These results are parallel to the effect of the extract used in this study on the LPO levels in tissues and they show that the plant species used in the study has positive effects on the activities of antioxidant molecules as well as protective effects against LPO.

Key words: *Pistacia terebinthus*, in vivo, enzymes, lipid peroxidation, glutathione, total protein

1. Introduction

Oxidative stress has been associated with aging and many chronic diseases such as atherosclerosis, diabetes, and cancer. Antioxidants could have a protective role in delaying or preventing oxidative stress, and much scientific research has focused on the antioxidant effects of foods, extracts, and pure compounds. Many compounds have been tested already in vitro to determine their antioxidant profiles (Cos et al., 2002, 2003). As these in vitro tests do not account for problems of malabsorption, distribution, metabolism, and excretion, it is imperative to investigate the activity of promising antioxidants in vivo.

Pistacia species (family Anacardiaceae) are widely distributed in Mediterranean countries. *Pistacia terebinthus* L. (turpentine tree, terebinth) is a perennial shrub or small tree widely growing in southern and western Turkey on dry rocky slopes and hillsides or in pine forests (Özcan et al., 2009). *P. terebinthus* fruits have been the subject of much research due to their antioxidant, antimicrobial (Topçu et al., 2007), and antiinflammatory properties (Giner-Larza et al., 2000) and their high oil contents (Matthaus and Ozcan, 2006). *P. terebinthus* fruits have been commonly consumed as coffee prepared with milk, known as 'menengiç' coffee in Turkish (Baytop, 1984). *P. terebinthus* fruits are rich in oil (approximately 40%) containing high concentrations of unsaturated fatty acids and carotenoids, phenolic compounds and tocopherols, tannin and resinous substances, and dietary fiber (10%) (Özcan, 2004; Matthäus and Özcan, 2006).

On the basis of this information and the rich ingredients of *P. terebinthus*, this study was carried out in order to examine the effects of *P. terebinthus* on oxidative stress in living tissues. The aim was to detect the effects of the extract on tissues of the rats by analyzing the levels of lipid peroxidation, glutathione, protein, glutathione peroxidase enzyme, and superoxide dismutase enzyme after oxidative stress was exerted in rats.

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2. Materials and methods

2.1. Experimental animals

Eight-week-old albino male Wistar rats of an inbred family were obtained from the Fırat University Experimental Research Center (FÜDAM) where the experiment was carried out. The rats were fed in a specially designed room, which was cleaned every day. The food was given in special steel containers and tap water was provided in bottles with stainless steel ball bearings. The animals used in the experiment were fed with special rat food pellets produced at the Elazığ Feed Factory. The contents of the food given to animals used in the experiment were as follows: wheat 10%, corn 21%, barley 14%, bran 8%, soybean meal 25%, fishmeal 8%, bone meal 4%, molasses 4%, salt 4%, a mixture of vitamins (A, D3, E, K, B1, B2, B6, and B12 vitamins with nicotinamide, folic acid, D biotin, and choline chloride) 1%, and a mixture of minerals (manganese, iron, zinc, copper, iodine, cobalt, selenium, and calcium) 1%.

Rats were divided into 4 groups according to their live weight. The following groups were used: 1) negative control: the rats were fed with a standard diet and were not given plant extract or hydrogen peroxide; 2) *Pistacia terebinthus* group (PT): the rats were fed with a standard diet and the plant extract was given but hydrogen peroxide was not given; 3) hydrogen peroxide group (HP): the rats were fed with a standard diet and were given hydrogen peroxide (a dose of 20 mg/kg live weight; 4) hydrogen peroxide + *P. terebinthus* group (PH): the rats were given both hydrogen peroxide and plant extract. The groups were organized taking the live weights of the rats into consideration, and the weights were equal in the beginning.

In order to exert oxidative stress in rats, an intraperitoneal injection of 20 mg/kg hydrogen peroxide (H_2O_2) was applied twice a week. Extracts (*P. terebinthus* flower) were produced by rending the sample plant group in solvents in a blender. After the rending process in the blender, all of the groups were centrifuged (5000 rpm, 4 °C). Processed plant extracts were administrated in a dose of 250 mg/kg every other day by gavage. The plants were extracted at a rate of 50/250 water. At the end of these applications, the rats were decapitated; samples of tissues and serums were gathered and kept until the analysis.

2.2. Plant materials and extraction procedures

Pistacia terebinthus flowers were collected from Işıklı village of Gaziantep Province, Turkey (37°09.415′N, 37°12.864′E, 1090 m a.s.l.) in the spring of 2009. The voucher specimens were deposited in the herbarium of the Department of Biology, Fırat University, Elazığ, Turkey. The taxonomic identification of plant materials was determined using *Flora of Turkey* (Davis, 1975). The aerial parts of the plant material were dried in shade at room temperature and then ground to a fine powder in a mechanic grinder. The powdered plant materials (50 g) were then extracted with 250 mL of water in a Soxhlet extractor (Gerhardt Soxtherm SOX-402, Germany). After the filtration of the solvent, the organic phases were independently concentrated under vacuum by evaporation to dryness. The dried extracts were dissolved in water to a final concentration of 100 mg/mL.

2.3. Gathering of tissue samples

Samples of blood, liver, kidneys, muscle, lungs, brain, heart, and spleen were gathered immediately after the decapitation of the rats. These parts of tissues were washed with 0.9% physiological saline solutions in order to clean the blood in tissues. The tissue samples were kept at –60 °C until biochemical processing.

2.4. Measuring the amount of lipid peroxidation (LPO) in tissues

Formation of malondialdehyde in an in vitro environment was expressed as thiobarbituric acid reactive substances (TBARS) calculated from a calibration curve using 1,1,3,3-tetraethoxypropane as the standard. Tissues were centrifuged with Tris-HCl and EDTA (pH 7) buffer at 4 °C at 9000 rpm for 10 min after homogenization. After taking 2 mL of the obtained supernatants, 6 mL of 0.084 N H_2SO_4 and 3 mL of 10% PHA were added; tissues were kept at room temperature for 5 min and supernatant liquid was removed by centrifuging at 5000 rpm for 10 min. After dissolving by adding 1 mL of distilled water to the remainder of the pellet, 1 mL of 3% hydrochloric acid and 1 mL of 0.6% TBA were added and tissues were kept at 95 °C for 60 min. After the process, 3 mL of butanol was added to cooled samples. The measurement was made by transferring 1 mL of supernatants into vials after centrifuging. The MDA-TBA (malondialdehyde-2-thiobarbituric acid) complex was analyzed using HPLC equipment. The equipment consisted of a pump (LC-10 ADVP), a fluorescence detector (RF-10 XL), a column oven (CTO-10ASVP), an autosampler (SIL-10ADVP), a degasser unit (DGU-14A), and a computer system with class VP software (Shimadzu, Kyoto, Japan). An Inertsil ODS-3 column (15 × 4.6 mm, 5 µm) was used as the HPLC column. The column was eluted isocratically at 20 °C with a 5 mM sodium phosphate buffer (pH 7.0) and acetonitrile (85:15, v/v) at a rate of 1 mL/min (De Las Heras et al., 2003).

2.5. Measuring the amount of glutathione in tissues

The samples of tissues gathered from the experimental animals in sufficient amounts were split from tissue pellets by centrifuging at 4 °C at 9000 rpm for 10 min after homogenization with Tris-HCl and EDTA (pH 7) buffer. At the end of centrifuging, the supernatant layer was taken and 1 mL of 5% TCA solution was added. This process enabled proteins to sediment. The obtained mixture was centrifuged (Refrigerated Centrifuge, Hettich 320R, UK) at 4500 rpm for 5 min; the pellet was settled and then the

supernatant layer was transferred to another tube. Then 1 mL of DTNB and 2 mL of 0.3 M Na_2HPO_4 solutions were added to the obtained supernatant (Elman, 1959); the resulting yellow color was read at 412 nm against the blank.

GSH quantities in samples were calculated according to a calibration curve based on pure GSH standard (Merck) ($y = 0.012x - 0.0043$, $R^2 = 0.9929$). For this reason, 0.002 g of pure glutathione for 10 mL was prepared and the groups were formed. GSH amounts in tissue pellets were measured in mg/g cell pellet.

2.6. Measuring the amount of total protein in tissues

Measurement of total protein amounts in tissues was done according to the Lowry method (Lowry et al., 1951). The reaction was read by spectrophotometer at 750 nm against the blank. For this purpose, 0.003 g of pure albumin for 10 mL was prepared and the groups were formed. The amounts of protein in the samples were calculated according to the equation for this calibration curve ($y = 0.0035x - 0.0237$, $R^2 = 0.9974$).

2.7. The measurement of glutathione peroxidase (GSH-Px) enzyme activity

The measurement of GSH-Px enzyme activity was done according to the Paglia and Valentine method (Paglia and Valentine, 1967). The basis of the GSH-Px enzyme measurement method is calculating the enzyme activity of GSH-Px by monitoring the decreasing absorbance of NADPH at 340 nm by spectrophotometer (Shimadzu UV-1700 Spectrophotometer, Japan) in a standardized experiment environment.

2.8. The measurement of superoxide dismutase

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured according to the method of Sun et al. and modification was made based on the method of Durak et al. (Sun et al., 1988; Durak et al., 1993). When SOD is in the environment, there is no reduction of NBT; a blue-purple color does not occur and a light color occurs depending on enzyme activity and amount.

2.9. Data Analysis

SPSS 15.0 was used for data analysis. The comparison between the experimental group and the control group was made using variance analysis (ANOVA) and LSD tests. The results were presented as mean ± SEM. $P < 0.01$, $P < 0.05$, $P < 0.001$, and $P < 0.0001$ values were used in differences between groups.

3. Results

3.1. The effect of plant extract on formation of lipid peroxidation in tissues

Levels of MDA, which is the last product of LPO in tissues, were measured. When the control group was compared to the HP group and PH group, it was observed that LPO levels in the liver decreased significantly ($P < 0.0001$). There were no meaningful differences when the PT (*Pistacia terebinthus*) group was compared to the control group. The LPO level in kidney tissue increased in the HP group ($P < 0.0001$), whereas it decreased in the PT and PH groups ($P < 0.0001$) when they were compared to the control group. It was found that especially MDA-TBA levels in the PH group significantly decreased. Compared to the control group, in muscle tissue, the LPO level in the PT group showed an insignificant decrease; the MDA-TBA level in the HP and PH groups decreased more significantly and the most significant decrease was detected in the PH group ($P < 0.0001$). LPO in the brain decreased dramatically ($P < 0.0001$), and the most dramatic decrease was in the PT group. In spleen tissue, LPO levels increased in all groups ($P < 0.0001$) as compared to the control group. Compared to the control group, in lung tissue, there were no differences in the HP and PT groups, while MDA-TBA level in the PH group decreased significantly ($P < 0.0001$) (Table 1).

3.2. The effects of plant extract on glutathione amounts in experimental animals

When glutathione amounts in tissues were examined, it was found that the glutathione amounts in the liver increased in

Table 1. The effect of plant extract on the amounts of lipid peroxidation in tissues (nmol/mL).

MDA (nmol/mL)	Control	PT	HP	PH
Liver	4.3 ± 0.08	4.1 ± 0.07	3.47 ± 0.14^{cd}	3.6 ± 0.07^{cd}
Kidney	4.6 ± 0.2	3.6 ± 0.14^{cd}	5.4 ± 0.3^{cd}	0.5 ± 0.02^{cd}
Muscle	30.6 ± 0.2	29.2 ± 0.6	25.4 ± 1.5^{cd}	24.9 ± 0.2^{cd}
Brain	2.8 ± 0.18	0.49 ± 0.03^{cd}	1.2 ± 0.07^{cd}	0.54 ± 0.03^{cd}
Spleen	81.9 ± 2.4	106.3 ± 2.2^{cd}	95.9 ± 1.5^{cd}	113.8 ± 2.7^{cd}
Lung	46.3 ± 2.1	46.5 ± 0.8	45.8 ± 1.7	32.4 ± 1.5^{cd}

cd: $P < 0.0001$.

the PT and the PH groups and decreased in the HP group ($P < 0.0001$) when compared to the control group. For the HP group, when the PT and PH groups were compared, it was discovered that glutathione level increased in the groups to which plant extract had been given, and the increase was more significant in the PT group ($P < 0.0001$). While glutathione level in the kidneys increased outstandingly in all other groups ($P < 0.0001$) as compared to the control group, the most outstanding increase was detected in the PH group. Compared to the control group, glutathione level in the brain meaningfully decreased in all groups ($P < 0.0001$). Compared to the control group, in muscle, there was no meaningful difference in the PT and HP groups, and there was a significant increase in the HP group ($P < 0.05$) in terms of glutathione levels. Compared to the control group, glutathione level in the heart did not show any significant differences in the HP and PT groups, but it significantly increased in the PH group ($P < 0.0001$). Compared to the control group, no significant differences were detected in glutathione levels in the spleen in the HP and the PT groups, but the glutathione level in the spleen significantly decreased in the PH group ($P < 0.0001$). Compared to the control group, in the lungs, glutathione level did not demonstrate any significant differences in the PH and PT groups, but it significantly decreased in the HP group ($P < 0.0001$) (Table 2).

3.3. The effects of plant extract on amounts of total protein in experimental animals

When total protein amounts in tissues were examined, it was observed that total protein level in the liver decreased in the HP group ($P < 0.0001$) and significantly increased in the PT and PH groups ($P < 0.0001$) as compared to the control group. Compared to the control group, in kidneys, the total protein level decreased in the HP group ($P < 0.0001$) and significantly increased in the PT group as well as the PH group, which showed more increase ($P < 0.0001$). It was also found that total protein level in muscle decreased in the HP and PT groups ($P < 0.0001$), whereas it significantly increased in the PH group ($P < 0.0001$) compared to the control group. Total protein level in the brain significantly increased in all groups ($P < 0.0001$) and the PT group ($P < 0.0001$) showed the most significant increase. Total protein level in all groups also significantly increased in heart tissue ($P < 0.0001$) and the most significant increase was observed in the PH group ($P < 0.0001$). Compared to the control group, total protein level in the spleen significantly decreased in all groups ($P < 0.0001$) and the significance levels were found as follows: the HP group ($P < 0.01$), the PT and the PH groups ($P < 0.0001$). While a significant decrease was obtained in total protein level of the HP group in lungs ($P < 0.0001$), there were no differences in the PT and PH groups in terms of total protein level in lungs (Table 3).

Table 2. The effect of plant extract on glutathione level in tissues (mg/g).

GSH (mg/g)	Control	PT	HP	PH
Liver	429.85 ± 8.7	492.9 ± 5.3^{cd}	292 ± 3.1^{cd}	471.7 ± 2.3^{cd}
Kidney	166.5 ± 2.5	440.4 ± 2.18^{cd}	300.8 ± 2.4^{cd}	537.9 ± 4.9^{cd}
Muscle	63.3 ± 0.89	64.1 ± 0.53	60.8 ± 0.25^{a}	63.7 ± 0.2
Heart	45.7 ± 0.3	46.9 ± 0.2	46.3 ± 0.7	54.6 ± 0.5^{cd}
Brain	33.3 ± 0.8	25 ± 0.4^{cd}	30.05 ± 0.15^{cd}	26.5 ± 0.3^{cd}
Spleen	48.4 ± 1.9	48.2 ± 1.2	50.7 ± 1.6	40.7 ± 1.2^{cd}
Lung	14.8 ± 0.3	14.7 ± 0.25	10.9 ± 0.29^{cd}	14.8 ± 0.29

cd: $P < 0.0001$, a: $P < 0.05$.

Table 3. The effect of plant extract on the total protein amounts in tissues (mg/g).

Total protein (mg/g)	Control	PT	HP	PH
Liver	80.6 ± 0.4	90.2 ± 0.7^{cd}	70.7 ± 0.5^{cd}	90.5 ± 0.16^{cd}
Kidney	100 ± 1.5	123.9 ± 0.7^{cd}	84.6 ± 0.4^{cd}	120.9 ± 2.4^{cd}
Muscle	37.1 ± 0.5	29.4 ± 0.18^{cd}	33.9 ± 0.3^{cd}	38.8 ± 0.3^{d}
Heart	57.9 ± 0.2	64.5 ± 0.2^{cd}	64.6 ± 0.2^{cd}	66.6 ± 0.3^{cd}
Brain	16.4 ± 0.13	18.4 ± 0.06^{cd}	17.4 ± 0.13^{cd}	17.3 ± 0.17^{cd}
Spleen	60.5 ± 1	53.1 ± 1.6^{cd}	54.2 ± 2.4^{c}	51.2 ± 0.6^{cd}
Lung	26.3 ± 0.26	25.3 ± 0.4	23.03 ± 0.14^{cd}	27.2 ± 0.45

cd: $P < 0.0001$, d: $P < 0.001$.

3.4. The effect of plant extract on enzyme activities in blood

Compared to the control group, there were no meaningful differences in the HP and PT groups in terms of GSH-Px enzyme activity, while GSH-Px enzyme activity significantly increased in the PH group ($P < 0.0001$).

Compared to the control group, SOD activity in blood significantly increased in the PT group ($P < 0.05$) and the PH group ($P < 0.0001$), but no differences appeared in the HP group (Table 4).

4. Discussion

It was observed that the plant extract used in this study had effects on different parameters in different tissues of Wistar rats. This plant's effects on preventing oxidative stress exerted in tissues via LPO were primarily investigated. It was found that the *P. terebinthus* plant had protective effects against LPO in the kidneys, brain, and lungs. It is thought that these effects are especially the results of phytochemical compounds that the plant extract contains. The effects of *P. terebinthus* extract on glutathione and total protein levels in tissues were also investigated, as well as the positive effects of *P. terebinthus* extract on LPO. The present study shows that *P. terebinthus* extract leads to an increase in and has positive effects on glutathione and total protein levels in all tissues except the brain and spleen. The results are parallel to the effects of the extract used in this study on LPO levels in tissues and show that the plant extract used also had positive effects on the activities of antioxidant molecules, in addition to preventive effects against LPO.

The effects of phytochemicals on increasing antioxidant molecules' activity and protective effects on similar oxidative stress exerted in tissues used in the experimental stage of this study were stated in many herb-based studies, which support our view. In Özşahin's study, it was found that the extract of apricots and grapes had protective effects against LPO exerted in the serum, erythrocytes, liver, kidneys, and brain. It was also detected in Özşahin's study that the extract of apricot and grapes leads to an increase in levels and has positive effects on all tissues except erythrocytes (Özşahin, 2010).

Konyalioglu and Karamenderes (2005) investigated the effects of *Achillea* (known as yarrow or milfoil), which naturally grows in Turkey, on oxidative stress induced with H_2O_2 in human erythrocyte and leukocytes. They found that *Achillea* species are natural antioxidant sources in preventing LPO. They also showed that these species remarkably increase GSH levels in erythrocytes and leukocytes as a result of the flavonoids and total phenols they contain. Bhatia and Jain (2004) reported that in oxidative stress conditions, spinach plant has reducing effects on LPO and GSH in the rat liver.

It has been observed that certain estrogen metabolites are involved in carcinogenesis where H_2O_2 plays an important role. H_2O_2 has been associated with the induction of cancer and has hence been reported as mutagenic (Shamberger, 1972; Pryor, 1986). In the present study, we have estimated LPO in terms of quantifying MDA levels. The lipid peroxidation products of PUFAs are considered to be important for genotoxic effects (Comporti, 1989; Esterbauer et al., 1990; Pryor and Porter, 1990; Eder et al., 2008). Various reactive oxygen species can induce LPO, whereas peroxidative fatty-acid fragments and their radicals can lead to formation of reactive oxygen species (Eder et al., 2008). Thus, MDA and 4-hydroxy-2-nonenal (HNE) are the two most prominent LPO products (Esterbauer et al., 1990).

Miura et al. (2003) found that serum LPO levels were significantly decreased by resveratrol. Skrzydlewska et al. (2001) investigated the protective effect of green tea against LPO in serum, brain, and liver tissues of rats. They found that green tea increases the levels of glutathione peroxidase and glutathione reductase in the liver whereas it reduces LOOH, 4-HNE, and MNA, which are markers of LPO, as well as leading to a decrease in glutathione levels in the liver. They also found that although green tea slightly increases the glutathione level in the serum it significantly decreases the MDA level. They argued that the damages in different tissues caused by LPO result differently because of the changes in the biomembranes of these tissues. They also reported that these positive results of the plant on tissues are the result of phytochemical compounds such as flavonoids the plants contain. They concluded that these polyphenols take effect more quickly in tissues rather than serum because they have a high rate of absorption in the gastrointestinal system.

The findings of the present study support other studies. In this study it was found that the glutathione level decreased in the liver, muscle, brain, and lung tissues of the groups to which H_2O_2 had been given. The

Table 4. The effect of plant extract on enzyme activities in blood (U/mL).

U/mL	Control	PT	HP	PH
GSH-Px	0.24 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.35 ± 0.006[cd]
SOD	6.92 ± 0.4	8.6 ± 0.5[a]	6.8 ± 0.2	10.4 ± 0.8[cd]

cd: $P < 0.0001$, a: $P < 0.05$.

glutathione levels in liver, kidney, muscle, and heart tissues increased in the groups to which plant extract had been given. This increase in glutathione levels is thought to be an adaptation mechanism against H_2O_2 (Türkoğlu, 2011). Glutathione is the most important step in defending against oxidative stress (Ahmed et al., 2000). GSH levels increase as a result of adaptation mechanisms that appear when oxidative stress is weak. Nevertheless, the GSH level decreases as a result of weakening adaptation mechanisms and increasing formation of GSSG when oxidative stress is strong (Zhang et al., 2005).

The effects of plant extract on GSH-Px and SOD enzyme activities in blood were also investigated in this study. It was found that GSH-Px enzyme activity significantly increased in the PH group and SOD enzyme activity meaningfully increased in the PT and PH groups. Hydrogen peroxide and superoxide ions, which are produced in plasma and which can go through erythrocyte cell membranes, are gathered in erythrocytes (İlhan, 1998). Erythrocytes are continuously exposed to oxidative stress. The reduction potential of a normal erythrocyte is 250 times more than its promoting potential (Gerber et al., 1991). In this respect, erythrocytes are important radical pools and prevent the formation of more toxic hydroxyl radicals by activating intracellular antioxidant defense systems such as GSH-Px and CAT for detoxification of hydrogen peroxide and SOD for dismutation of superoxide ion radicals (İlhan, 1998). SOD, which catalyzes the transformation of superoxide anion radical into hydrogen peroxide and molecular oxygen, is an antioxidant metalloproteinase. Mn-SOD localizes in the mitochondrial matrix. Mn-SOD features in the protection against oxidative cell damage and regulation of cellular concentration of O_2, which is a product of cellular metabolism and a highly reactive oxidant. It is stated that Mn-SOD expression is low in many tumor cells. Overexpression of Mn-SOD in human melanoma cells, breast cancer cells, and glioma cells is thought to be due to a tumor suppressor gene that suppresses formation of tumors (Mukhopadhyay et al., 2004).

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